



Europäisches Patentamt

European Patent Office

Office européen des brevets

Rec'd PCT/PTO 10 JUN 2005

(11) EP 0 983 769 A1

10/538686

(12) **EUROPEAN PATENT APPLICATION**
published in accordance with Art. 158(3) EPC

(43) Date of publication:
08.03.2000 Bulletin 2000/10

(21) Application number: 98912774.1

(22) Date of filing: 13.04.1998

(51) Int. Cl.⁷: A61K 47/14

(86) International application number:
PCT/JP98/01679

(87) International publication number:
WO 98/46269 (22.10.1998 Gazette 1998/42)

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU
MC NL PT SE

(30) Priority: 14.04.1997 JP 9561897

(71) Applicant:
Mitsubishi-Tokyo Pharmaceuticals, Inc.
Chuo-ku, Tokyo 103-8405 (JP)

(72) Inventors:
• YAZAKI, Takashi
Tokyo Tanabe Company Limited
Tokyo 103-8405 (JP)

• HASHIMOTO, Mitsuo
Tokyo Tanabe Company Limited
Tokyo 103-8405 (JP)
• NAKANISHI, Seiki
Tokyo Tanabe Company Limited
Tokyo 103-8405 (JP)

(74) Representative:
Bühling, Gerhard, Dipl.-Chem.
Patentanwaltsbüro
Tiedtke-Bühling-Kinne & Partner
Bavariaring 4
80336 München (DE)

(54) **PERMUCOUS PREPARATION**

(57) A permucous composition characterized by comprising Antago-3 or a physiologically acceptable salt thereof and a sucrose/fatty acid ester. The composition can serve as a pharmaceutical preparation which has the high permucous absorbability of physiologically active peptide Antago-3 and is unstimulative and stable for long.

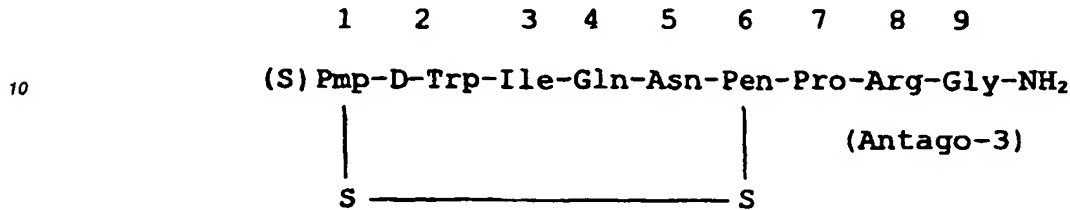
BEST AVAILABLE COPY

EP 0 983 769 A1

Description

Technical Field

5 [0001] The present invention relates to a composition containing a compound represented by the following formula:



Pmp: β,β -(3-thiapentamethylene)- β -mercaptopropionic acid

D-Trp: D-tryptophan

20 Pen: L-penicillamine

Ile: L-isoleucine Gln: L-glutamine

Asn: L-asparagine Pro: L-proline

Arg: L-arginine

25 (hereunder referred to as "Antago-3") or a physiologically acceptable salt thereof, and more specifically it relates to a permucosal preparation containing as an effective component Antago-3 or a physiologically acceptable salt thereof.

Background Art

30 [0002] Antago-3 is a synthetic peptide with a molecular weight of 1172.47, which is an oxytocin analog with a powerful and specific oxytocin antagonism effect (WO94/25485-A).

[0003] Physiologically active peptides readily undergo enzymolysis by peroral or permucosal administration, while they are virtually unabsorbed by percutaneous administration due to their low membrane permeability, and hence they are generally administered in the form of injections.

35 [0004] For permucosal administration of physiologically active peptides, expression of their drug effects has required a method whereby an enzyme inhibitor or protective substance is added to protect the drugs from enzymes, or a method whereby an absorption enhancer is added to promote absorption of the drugs before their degradation.

[0005] As permucosal preparations of physiologically active peptides there are known preparations containing cyclodextrin (Japanese Patent Publication No. 19092 of 1990), preparations containing bile acid (Japanese Patent Publication No. 25068 of 1994, Japanese Laid-open Patent Publications No. 2932 of 1988 and No. 194260 of 1993), preparations containing surfactants (Japanese Laid-open Patent Publications No. 130820 of 1984 and No. 185030 of 1987), preparations containing absorption accelerators (Japanese Laid-open Patent Publications No. 267528 of 1986 and No. 196524 of 1988), preparations containing sucrose fatty acid esters (Japanese Laid-open Patent Publications No. 39822 of 1988, No. 214 of 1990 and No. 101020 of 1990), preparations containing saccharides (Japanese Laid-open Patent Publication No. 502920 of 1991) and preparations containing ethylenediamine tetraacetate (Japanese Laid-open Patent Publication No. 306921 of 1990), but no permucosal preparation is known that contains Antago-3 or a physiologically acceptable salt thereof as an effective component.

Disclosure of Invention

50 [0006] When an aqueous solution (10 μ g/kg) of Antago-3 acetate (hereunder referred to simply as "Antago-3") was intravenously administered to rats, the response rate on uterine motility was 30% after 30 minutes, and the response rate was 5% after one hour.

[0007] However, with pernasal administration of the Antago-3 aqueous solution, it was not possible to express a similar drug effect with intravenous administration even at a dose of 400 μ g/kg.

55 [0008] When polyoxyethylene polyoxypropylene ether, lecithin, sodium caprylate, sodium caprate, aprotinin or bacitracin, sodium ethylenediamine tetraacetate, sodium deoxycholate, β -cyclodextrin, sodium salicylate or disodium glycyrrhizinate was added to the Antago-3 aqueous solution as an absorption enhancer, it was not possible to express the

drug effect obtained by intravenous administration with any of these other than sodium deoxycholate. Incidentally, sodium deoxycholate was shown to have an irritating effect on the nasal cavity mucosa.

[0009] The absorption enhancing effect of adding polysorbate 80, polyoxyethylene sorbitan monolaurate, taurine or N,N-dimethylacetamide (hereunder, "DMA") to the Antago-3 aqueous solution was evaluated based on the Antago-3 blood concentration and blood concentration area under the curve (hereunder, "AUC"), but no absorption enhancing effect was exhibited by any of the compounds.

[0010] For permucosal administration of an absorption enhancer-added Antago-3 liquid preparation, it is necessary that the presence of the additive cause no non-uniformity such as clouding or precipitation, or irritation at the site of administration.

[0011] The present inventors have conducted diligent research on permucosal preparations containing Antago-3 as the effective component, and as a result we have found that when a composition containing Antago-3 and a sucrose fatty acid ester (hereunder referred to as "composition of the invention") is administered, the Antago-3 is very efficiently absorbed through the nasal mucosa and rectal mucosa, thus allowing expression of a drug effect.

[0012] However, it was not easy to maintain a transparent state for an aqueous preparation containing the composition of the invention, due to clouding or precipitation of the sucrose fatty acid ester.

[0013] Upon conducting diligent research on Antago-3-containing aqueous preparations that maintain transparency, the present inventors then found, surprisingly, that the preservative benzalkonium chloride (hereunder, "BZCL") has a preventive effect against clouding and precipitation of the sucrose fatty acid ester while also causing virtually no mucosal irritation so that it is highly safe for the body, and the present invention has thus been completed.

[0014] The composition for permucosal administration provided by the invention comprises a sucrose fatty acid ester in a proportion of 0.25-50 parts by weight to one part by weight of Antago-3. The fatty acid of the sucrose fatty acid ester may be stearic acid, palmitic acid, myristic acid, lauric acid, etc., and esters, diesters and triesters of these fatty acids, or mixtures thereof, may be used.

[0015] The preparation form may be a solution preparation or other type of liquid preparation, or a powder preparation.

[0016] BZCL may be used as a preservative that can be added to the aqueous preparation as a solution preparation, and it is combined at 0.03-0.5 part by weight, and preferably 0.04-0.16 part by weight of BZCL to one part by weight of the sucrose fatty acid ester. As isotonicizing agents there may be mentioned polyols such as glycerin and propylene glycol, saccharides such as mannitol, and nicotinamide, and as viscosity agents there may be mentioned hydroxypropylcellulose (hereunder, "HPC"), polyvinylpyrrolidone (hereunder, "PVP"), carboxymethylcellulose sodium (hereunder, "CMC-Na") and polyvinyl alcohol (hereunder, "PVA").

[0017] A powder preparation may be administered by a method whereby a mixture of the composition of the invention with lactose, sucrose, mannitol, sorbitol, crystalline cellulose, starch or a low-substituted HPC is sprayed by air power, or a method of spraying with an appropriate volatile carrier.

[0018] After adding the preservative, viscosity agent, surfactant, etc. to the composition of the invention, it may be filled into a container and used as a rectal preparation or vaginal preparation. As preservatives there may be mentioned *p*-hydroxybenzoic acid ester, BZCL, chlorobutanol and sodium dehydroacetate, as viscosity agents, HPC, PVP, CMC-Na, PVA and carboxyvinyl polymer, and as surfactants, hydrogenated castor oil and macrogol.

Best Mode for Carrying Out the Invention

[0019] The present invention will now be described by way of production examples. These examples, however, are not intended to be restrictive on the invention.

(Comparative Example 1)

[0020] Antago-3 (10.0 mg) was dissolved in 100 mL of distilled water to make a 0.01% Antago-3 aqueous preparation.

(Comparative Examples 2-4)

[0021] According to the method of Comparative Example 1 there were prepared 0.4% Antago-3 (Comparative Example 2), 0.2% Antago-3 (Comparative Example 3) and 0.04% Antago-3 (Comparative Example 4) aqueous preparations.

(Comparative Example 5)

[0022] A 1 mL aqueous solution of 0.16% Antago-3 and a 1 mL aqueous solution of 1.0% polyoxyethylene polyoxypropylene ether were thoroughly mixed to make a 0.08% Antago-3 aqueous preparation containing 0.5% polyoxyethylene polyoxypropylene ether.

(Comparative Examples 6-20)

[0023] According to the method of Comparative Example 5 there were made 0.08% Antago-3 aqueous preparations containing 0.5% lecithin (Comparative Example 6), 0.5% sodium caprylate (Comparative Example 7), 0.5% sodium caprate (Comparative Example 8), 0.1% aprotinin (Comparative Example 9), 0.1% bacitracin (Comparative Example 10), 1.0% sodium ethylenediamine tetraacetate (Comparative Example 11) and 1.0% sodium deoxycholate (Comparative Example 12), a 0.04% Antago-3 aqueous preparation containing 0.5% sodium deoxycholate (Comparative Example 13), and 0.08% Antago-3 aqueous preparations containing 0.5% β -cyclodextrin (Comparative Example 14), 0.1% sodium salicylate (Comparative Example 15), 1.0% disodium glycyrrhizinate (Comparative Example 16), 0.5% polysorbate 80 (Comparative Example 17), 0.5% polyoxyethylene sorbitan monolaurate (Comparative Example 18), 1.0% taurine (Comparative Example 19) and 3.0% DMA (Comparative Example 20).

(Comparative Example 21)

[0024] After thoroughly mixing 1 mL of a 1.0% Antago-3 aqueous solution and 1 mL of a 1.0% sucrose fatty acid ester (stearic acid ester) solution, the mixture was lyophilized to obtain an Antago-3/sucrose fatty acid ester (1/1) composition for permucosal administration. To this there were added to dissolution 1 mL of a 5.0% glycerin aqueous solution and 1 mL of a 1.0% benzyl alcohol aqueous solution, to make an aqueous preparation of the composition for permucosal administration.

(Comparative Example 22)

[0025] The Antago-3/sucrose fatty acid ester (1/1) lyophilized composition for permucosal administration of Comparative Example 21 was dissolved in an aqueous solution prepared by adding distilled water to 1 mL of a 5.0% glycerin aqueous solution, 0.2 mL of a 0.2% methylparaben aqueous solution and 0.2 mL of a 0.4% propylparaben aqueous solution, for a total of 2 mL, to make an aqueous preparation of the composition for permucosal administration.

(Comparative Example 23)

[0026] Distilled water was added to 1 mL of a 5.0% Antago-3 aqueous solution and 1 mL of a 1.0% sucrose fatty acid ester (stearic acid ester) to a volume of 10 mL, to make an aqueous preparation of an Antago-3/sucrose fatty acid ester (1/0.2) composition for permucosal administration.

(Comparative Example 24)

[0027] Distilled water was added to a mixture of 1 mL of a 0.8% Antago-3 aqueous solution, 5 mL of a 1.0% sucrose fatty acid ester (stearic acid ester) aqueous solution, 2 mL of a 12.5% glycerin aqueous solution and 0.25 mL of a 0.4% BZCL aqueous solution to a volume of 10 mL to make an aqueous preparation of an Antago-3/sucrose fatty acid ester (1/6.25) composition for permucosal administration.

(Example 1)

[0028] After thoroughly mixing 1 mL of a 0.16% Antago-3 aqueous solution and 1 mL of a 1.0% sucrose fatty acid ester (stearic acid ester) aqueous solution, the mixture was lyophilized to obtain an Antago-3/sucrose fatty acid ester (1/6.25) composition for permucosal administration.

[0029] This was dissolved in 2 mL of distilled water to make an aqueous preparation of the composition for permucosal administration.

(Example 2)

[0030] After thoroughly mixing 1 mL of a 0.08% Antago-3 aqueous solution and 1 mL of a 1.0% sucrose fatty acid ester (palmitic acid ester) aqueous solution and lyophilizing the mixture, the resulting Antago-3/sucrose fatty acid ester (1/12.5) composition for permucosal administration was dissolved in 2 mL of distilled water to make an aqueous preparation of the composition for permucosal administration.

(Example 3)

[0031] After thoroughly mixing 1 mL of a 0.04% Antago-3 aqueous solution and 1 mL of a 1.0% sucrose fatty acid

ester (stearic acid ester and palmitic acid ester at weight ratio of 1:1) aqueous solution and lyophilizing the mixture, the resulting Antago-3/sucrose fatty acid ester (1/25) composition for permucosal administration was dissolved in 2 mL of distilled water to make an aqueous preparation of the composition for permucosal administration.

(Example 4)

[0032] After thoroughly mixing 1 mL of a 0.04% Antago-3 aqueous solution and 1 mL of a 0.2% sucrose fatty acid ester (stearic acid ester) aqueous solution and lyophilizing the mixture, the resulting Antago-3/sucrose fatty acid ester (1/5) composition for permucosal administration was dissolved in 2 mL of distilled water to make an aqueous preparation of the composition for permucosal administration.

(Example 5)

[0033] After thoroughly mixing 1 mL of a 0.02% Antago-3 aqueous solution and 1 mL of a 1.0% sucrose fatty acid ester (stearic acid ester) aqueous solution and lyophilizing the mixture, the resulting Antago-3/sucrose fatty acid ester (1/50) composition for permucosal administration was dissolved in 2 mL of distilled water to make an aqueous preparation of the composition for permucosal administration.

(Example 6)

[0034] The same treatment was carried out except that the sucrose fatty acid ester (stearic acid ester) of Example 1 was replaced with another sucrose fatty acid ester (sucrose lauric acid ester), and the resulting Antago-3/sucrose fatty acid ester (1/6.25) composition for permucosal administration was dissolved in 2 mL of distilled water to make an aqueous preparation of the composition for permucosal administration.

(Example 7)

[0035] The Antago-3/sucrose fatty acid ester (1/6.25) composition for permucosal administration of Example 1 was dissolved in an aqueous solution prepared by adding distilled water to a mixed solution of 1 mL of a 5.0% nicotinamide solution and 0.2 mL of a 0.2% BZCL solution to a volume of 2 mL, to make an aqueous preparation of the composition for permucosal administration.

(Example 8)

[0036] The Antago-3/sucrose fatty acid ester (1/6.25) composition for permucosal administration of Example 1 was dissolved in an aqueous solution prepared by adding distilled water to a mixed solution of 1 mL of a 10.0% sorbitol aqueous solution and 0.2 mL of a 0.2% BZCL solution to a volume of 2 mL, to make an aqueous preparation of the composition for permucosal administration.

(Example 9)

[0037] The Antago-3/sucrose fatty acid ester (1/6.25) composition for permucosal administration of Example 1 was dissolved in an aqueous solution prepared by adding distilled water to a mixed solution of 1 mL of a 5.0% glycerin aqueous solution and 0.2 mL of a 0.2% BZCL solution to a volume of 2 mL, to make an aqueous preparation of the composition for permucosal administration.

(Example 10)

[0038] Distilled water was added to 1 mL of a 0.8% Antago-3 aqueous solution, 1 mL of a 5% sucrose fatty acid ester (stearic acid ester) aqueous solution, 2 mL of a 12.5% glycerin aqueous solution, 1 mL of a 0.1% methylparaben-0.2% propylparaben mixed aqueous solution, 3 mL of a 10% HPC aqueous solution and 1 mL of a 0.2% BZCL aqueous solution to a volume of 10 mL to make an aqueous preparation of an Antago-3/sucrose fatty acid ester (1/6.25) composition for permucosal administration.

(Example 11)

[0039] Distilled water was added to 1 mL of a 0.8% Antago-3 aqueous solution, 1 mL of a 5% sucrose fatty acid ester (stearic acid ester) aqueous solution, 2 mL of a 12.5% glycerin aqueous solution, 1 mL of a 5% benzyl alcohol aqueous

solution, 3 mL of a 10% HPC aqueous solution and 1 mL of a 0.2% BZCL aqueous solution to a volume of 10 mL to make an aqueous preparation of a composition for permucosal administration.

(Example 12)

[0040] After adding 10 mL of a 1.0% sucrose fatty acid ester (stearic acid ester) solution, 9.2 g of 2% Hibis Waco 105 (trade name: Wako Junyaku) as a gel base and a 1 mol/L sodium hydroxide aqueous solution (0.8 g) to Antago-3 (16.0 mg), the total was brought to 20 g with distilled water and thoroughly mixed to make a gel preparation of an Antago-3/sucrose fatty acid ester (1/6.25) composition for permucosal administration.

(Example 13)

[0041] Distilled water was added to 1 mL of a 5.0% Antago-3 aqueous solution, 5 mL of a 1.0% sucrose fatty acid ester (stearic acid ester) aqueous solution, 2 mL of a 12.5% glycerin aqueous solution and 2 mL of a 0.4% BZCL aqueous solution to a volume of 10 mL to make an aqueous preparation of an Antago-3/sucrose fatty acid ester (1/1) composition for permucosal administration.

(Example 14)

[0042] Distilled water was added to 1 mL of a 0.8% Antago-3 aqueous solution, 5 mL of a 1.0% sucrose fatty acid ester (stearic acid ester) aqueous solution, 2 mL of a 12.5% glycerin aqueous solution and 2 mL of a 0.4% BZCL aqueous solution to a volume of 10 mL to make an aqueous preparation of an Antago-3/sucrose fatty acid ester (1/6.25) composition for permucosal administration.

(Example 15)

[0043] Distilled water was added to 1 mL of a 0.8% Antago-3 aqueous solution, 5 mL of a 1.0% sucrose fatty acid ester (stearic acid ester) aqueous solution, 2 mL of a 12.5% glycerin aqueous solution and 1 mL of a 0.4% BZCL aqueous solution to a volume of 10 mL to make an aqueous preparation of an Antago-3/sucrose fatty acid ester (1/6.25) composition for permucosal administration.

(Example 16)

[0044] An aqueous preparation of a composition for permucosal administration was made by the same procedure in Example 15 except that the 0.4% BZCL aqueous solution was replaced with a 0.2% BZCL aqueous solution.

(Example 17)

[0045] Distilled water was added to 1 mL of a 5.0% Antago-3 aqueous solution, 2 mL of a 2.0% sucrose fatty acid ester (stearic acid ester) aqueous solution, 2 mL of a 12.5% glycerin aqueous solution and 1 mL of a 0.2% BZCL aqueous solution to a volume of 10 mL to make an aqueous preparation of an Antago-3/sucrose fatty acid ester (1/0.8) composition for permucosal administration.

(Example 18) (Preparation 1 for monkey)

[0046] Distilled water was added to 50 mg of Antago-3, 4 mL of a 2.5% sucrose fatty acid ester (stearic acid ester) aqueous solution, 2 mL of a 0.4% BZCL aqueous solution and 2 mL of a 12.5% glycerin aqueous solution to a volume of 20 mL to make an aqueous preparation of an Antago-3/sucrose fatty acid ester (1/2) composition for permucosal administration.

(Example 19) (Preparation 2 for monkey)

[0047] An aqueous preparation of an Antago-3/sucrose fatty acid ester (1/1) composition for permucosal administration was made by the same procedure in Example 18 except that 2 mL of the 2.5% sucrose fatty acid ester (stearic acid ester) aqueous solution was used.

(Example 20) (Preparation 3 for monkey)

[0048] An aqueous preparation of an Antago-3/sucrose fatty acid ester (1/0.4) composition for permucosal administration was made by the same procedure in Example 18 except that 0.8 mL of the 2.5% sucrose fatty acid ester (stearic acid ester) aqueous solution was used.

(Example 21) (Preparation 4 for monkey)

[0049] An aqueous preparation of an Antago-3/sucrose fatty acid ester (1/0.5) composition for permucosal administration was made by the same procedure in Example 18 except that 100 mg of Antago-3 was used and 2 mL of the 2.5% sucrose fatty acid ester (stearic acid ester) aqueous solution was used.

(Example 22) (Preparation 5 for monkey)

[0050] An aqueous preparation of an Antago-3/sucrose fatty acid ester (1/0.25) composition for permucosal administration was made by the same procedure in Example 18 except that 200 mg of Antago-3 was used and 2 mL of the 2.5% sucrose fatty acid ester (stearic acid ester) aqueous solution was used.

(Example 23)

[0051] A 10 mg portion of an Antago-3/sucrose fatty acid ester (1/1) lyophilized composition for permucosal administration produced according to Comparative Example 21 was thoroughly mixed with 290 mg of lactose. A hard capsule (#4) was filled with 30 mg of the mixture to make a powder preparation of the composition for permucosal administration.

(Example 24)

[0052] A powder preparation of a composition for permucosal administration was made according to the same procedure as Example 23, except that the lactose was replaced with crystalline cellulose.

(Example 25)

[0053] After adding 10 mL of a 1.0% sucrose fatty acid ester (palmitic acid ester) solution, 9.2 g of 2% Hibis Waco 105 (trade name: Wako Junyaku) as a gel base and a 1 mol/L sodium hydroxide aqueous solution (0.8 g) to Antago-3 (16.0 mg), the total was brought to 20 g with distilled water and thoroughly mixed to make a gel preparation of an Antago-3/sucrose fatty acid ester (1/6.25) composition for permucosal administration.

(Example 26)

[0054] A gel preparation of an Antago-3/sucrose fatty acid ester (1/6.25) composition was made by the same procedure as Example 25, except that the sucrose fatty acid ester (palmitic acid ester) was replaced with another sucrose fatty acid ester (myristic acid ester).

[Uterine motility response rate]

(Method)

[0055] Estrus was induced in female rats (8 weeks old) with estradiol prior to the experiment. With each rat under urethane anesthesia, a catheter was inserted into the uterine horn, and after injection of physiological saline it was connected to a low-pressure transducer. A distortion measurement amplifier was used to record a rectigraph of uterine motility. After continuous intravenous infusion of oxytocin (50 mU/kg/min) through the femoral vein and confirmation of a stabilized contraction waveform, the test sample was perinasally administered (100 μ L/kg). The suppressing effect on uterine motility by Antago-3 was expressed by the response rate, where 100% was defined as the multiplier of the average contraction frequency and amplitude in 10 minutes for the contraction waveform with oxytocin alone prior to administration of the sample. A response rate on uterine motility closer to 0% indicates that a stronger effect of Antago-3 was exhibited.

(Results)

[0056] The response rates on uterine motility with pernasal administration of Antago-3 aqueous solutions and aqueous solutions of the compositions of the invention are shown in Table 1, with intravenous administration of Antago-3 used as a control.

Table 1

Response rates on uterine motility with pernasal administration											
Sample	Dosage (μg/kg)		Response rate (%) Time (hr)								
			0.5	1.0	1.5	2.0					
Comparative Example	1	2	10	400	30	80	5	25	-20	- (intravenous injection)	20
	2			400		80		25			20
	3			200		90		70			50
	4			40		100		100			100
	5			80		100		100			100
	6			80		100		100		-	100
	7			80		100		100		-	100
	8			80		90		50		-	40
	9			80		100		100		100	100
	10			80		100		100		100	100
	11			80		100		100		100	100
	12			80		0		0		0	10
	13			40		30		20		15	10
	14			80		100		100		80	40
	15			80		80		80		80	80
	16			80		100		90		80	75
Example	1			80		0		0		0	0
	2			40		5		0		0	0
	3			20		10		5		5	5
	4			20		30		10		10	10
	5			10		30		15		10	5

[0057] With intravenous administration of the Antago-3 aqueous solution, uterine motility was inhibited with a dosage of 10 $\mu\text{g/kg}$. With pernasal administration of the Antago-3 aqueous solution, a dosage of at least 400 $\mu\text{g/kg}$ was required to obtain the same drug effect as the intravenous administration.

[0058] The sucrose fatty acid esters had a notable effect of enhanced Antago-3 absorption, with the aqueous solutions of the composition of the invention exhibiting activity roughly equivalent to that observed with intravenous administration.

[0059] No absorption enhancing effect was exhibited and uterine motility could not be inhibited when polyoxyethylene polyoxypropylene ether, lecithin, sodium caprylate, sodium caprate, aprotinin, bacitracin, sodium ethylenediamine tetraacetate, β -cyclodextrin, sodium salicylate or disodium glycyrrhizinate was added to Antago-3 as an absorption enhancer. When sodium deoxycholate was added, an absorption enhancing effect equivalent to that of the composition of the invention was exhibited.

[Absorption enhancement by blood concentration AUC method]

(Method)

(1) Pernal administration

[0060] A 100 $\mu\text{L/kg}$ dose of the test sample was administered into both nasal cavities of awake 9-week-old female rats on a 16-hour fast, and 0.2 mL of blood was taken from the cervical vein at 15 minutes, 30 minutes, 1 hour, 2 hours and 4 hours after administration. The blood was transferred to an opaque blood collecting tube, and an inhibitor (a solution prepared by dissolving 100,000 KIU of aprotinin (product of Wako Junyaku) and 4 mg of bestatin (product of Sigma Co.) in 5 mL of distilled water at the time of use) in an amount of 1/20 the amount of blood was silently added along the wall of the collecting tube, after which the blood was held in ice water and within 1.5 hours was centrifuged (4°C, 1600 \times g, 15 minutes) to obtain the blood plasma which was stored at -80°C until measurement. The Antago-3 in the plasma was measured by radioimmunoassay (RIA).

(2) Perrectal administration

[0061] The anus was held open with tweezers and the test sample was injected at 100 $\mu\text{L/kg}$ in solution form or 100 $\mu\text{g/kg}$ in gel form using an injection tube or a pipette, after which the anus was closed off with an adhesive to prevent leakage of the test sample. Blood was taken and measured in the same manner as for the pernasal administration.

[0062] The blood concentration area under the curve (AUC) was determined by the trapezoid method, and the absorption enhancement was expressed as the ratio to the AUC for the sample of (Comparative Example 2) in which no absorption enhancer was added. The test sample wherein 80 $\mu\text{g/kg}$ of Antago-3 was administered was evaluated based on the value of 5 times the calculated AUC value for comparison with Comparative Example 2 (400 $\mu\text{g/kg}$ administration).

(Results)

[0063] The results are shown in Tables 2 and 3.

Table 2

Blood concentration, AUC and absorption enhancement (pernasal administration)								
Sample		Blood concentration (ng/mL) Time (hr)					AUC _{0→4hr} (ng · hr/mL)	Absorption enhancement
		0.25	0.5	1.0	2.0	4.0		
Comparative Example	2	3	2	2	2	1	7	1
	17	1	0	0	0	0	-	-
	18	7	3	0	0	0	3	2
	19	1	0	0	0	0	-	-
	20	1	0	0	0	0	-	-
Example	1	60	30	15	5	1	46	33
	6	55	25	15	3	1	38	27
	7	65	35	17	5	1	51	36
	8	55	25	15	3	1	38	27
	9	60	35	15	3	1	44	31
	10	50	20	10	3	1	31	22
	11	55	30	15	5	1	45	32

Table 3

Blood concentration, AUC and absorption enhancement (perrectal administration)								
Sample		Blood concentration (ng/mL) Time (hr)					AUC _{0→4hr} (ng · hr/mL)	Absorption enhancement
		0.25	0.5	1.0	2.0	4.0		
Comparative Example	2	22	15	9	5	2	27	1
Example	1	35	27	15	10	1	46	9
	12	42	27	13	5	1	39	7

[0064] The absorption enhancements of the aqueous preparations and gel preparations of compositions according to the invention were 22-fold to 36-fold with pernasal administration and 7-fold to 9-fold with perrectal administration, compared to those with no addition of an absorption enhancer.

[Stability of solution preparations]

(Method)

[0065] Each of the solution preparations (Examples 13-17) made from a composition of the invention was placed in a transparent stoppered glass test tube, and stored at 5°C. Appearance were evaluated by visually observing the solution state at 1000 lux under a white fluorescent lamp.

(Results)

[0066] The preparations in which benzyl alcohol (Comparative Example 21) and methylparaben/propylparaben (Comparative Example 22) were added as preservatives, the preparation in which no preservative was added (Comparative Example 23) and the preparation with low addition of BZCL (Comparative Example 24) exhibited clouding after 3 days of storage, due to precipitation of the sucrose fatty acid esters. However, the preparations of Examples 13-17, in which at least 0.04 part by weight of BZCL was added to one part by weight of the sucrose fatty acid ester, maintained a colorless, transparent state.

[Nasal mucosa irritation]

(Method)

[0067] Eight-week-old female rats were used, with five per group. The rats were administered 100 µL/kg of the test sample into the nasal cavities once a day, for 7 continuous days. On the 8th day the nasal cavities were extracted, and after dividing them into 3 parts from the nasal cavity opening to the deep end of the nasal cavity, they were fixed with formalin to prepare specimens. After 10-14 days of fixing, a histopathological examination was made of the state of the mucosa, exudation and any bleeding.

[0068] Mild irritation was judged when abnormalities were found in the nasal mucosa at sections near the nasal cavity-opening, and moderate to severe irritation was judged when the sections where abnormalities were found were further to the deep end. Moderate or greater irritation was also judged when degeneration, ablation, exudation or bleeding was found on the nasal mucosa epithelium at sections near the nasal cavity opening.

[0069] As controls there were used physiological saline and commercially available desmopressin (Kyowa Hakko Kogyo, KK.).

(Results)

[0070] The results are shown in Table 4.

Table 4

Nasal cavity mucosa irritation					
		Normal	Mild	Moderate	Severe
Physiological saline		3	2	0	0
Desmopressin		5	0	0	0
Comparative Example	13	1	2	2	0
	21	4	1	0	0
Example	1	5	0	0	0
	2	5	0	0	0
	4	5	0	0	0
	15	4	1	0	0

[0071] In the [Comparative Example 13] group there were observed exudation in the nasal mucosa and beaker cell proliferation in the respiratory epithelium, and therefore moderate irritation was determined.

[0072] Beaker cell proliferation was found in the respiratory epithelium of one rat in each of the [Comparative Example 21] and [Example 15] groups, but it was milder than in the [physiological saline] group used as a control.

[0073] No irritation was found in the [Example 1] group, [Example 21 group, [Example 4] group or [desmopressin] group.

[0074] No dead animals were found in any of the groups, nor were any abnormal symptoms found.

[Monkey absorption test]

[0075] A female monkey with a body weight of 3 kg was strapped to a monkey chair without anesthesia and in a state of rest, and the head was held so that the external naris was directed straight downward. A fixed-dose atomizing spray apparatus (spray volume: 50 μ L/puff) filled with the test solution was then held vertically upward, and after stabilizing the spray condition by test spraying 4 or 5 times, the tip (about 1 cm) was inserted into the right nasal cavity and sprayed once for 50 μ L. After spraying, the external naris was pointed forward and the external naris which had received the agent was closed with a finger and held for about 10 seconds in order to prevent run-off of the administered test solution, after which the left nasal cavity was sprayed in the same manner. The animal had been fasting from the evening of the day prior to administration, and it was fed after completion of the blood sampling during the 24 hours after administration.

[0076] For blood sampling, 0.3 mL of blood was taken from the femoral vein or anterior humeroradial lateral cutaneous vein at 5, 15 and 30 minutes, and 1 and 2 hours (total of 5 times) after administration for preparations 1, 3, 4 and 5, and at 5, 15 and 30 minutes, and 1, 2, 4, 8 and 24 hours (total of 8 times) after administration for preparation 2.

[0077] The sampled blood was processed in the same manner as for the pernasal administration to obtain blood plasma which was stored at -80°C until measurement.

[Measurement of blood concentration]

[0078] In an Isolute C18 cartridge (manufactured by Uniflex Co.) there were added 0.2 mL - 0.05 mL of blood plasma, 0.1 mL of an internal standard solution (70% methanol solution containing 0.04 ppm reserpine) and 0.5 mL of a 0.1 M phosphate buffer solution (pH 7.0) to adsorb the contaminants in the plasma, and the Antago-3 was eluted out with 2 mL of 1% acetic acid-containing methanol. The eluate was distilled under reduced pressure, and 0.2 mL of a methanol:1.0% acetic acid mixture (7:3) was added to and dissolved in the residue to prepare a test solution. Separately, a bulk agent for quantitation was treated in the same manner and used as a standard solution. The test solution and the standard solution were subjected to liquid chromatography to determine the blood concentration.

Table 5

Blood concentrations					
	Blood concentration (ng/mL)				
	5 min	15 min	30 min	1 hour	2 hours
Example 18	112	372	346	456	368
	±23	±105	±80	±52	±78
Example 19	286	491	419	313	407
	±111	±193	±105	±66	±107
Example 20	74	102	160	96	130
	±23	±105	±80	±52	±78
Example 21	385	1161	2087	2252	2424
	±152	±245	±651	±592	±692
Example 22	828	2118	4201	2903	2851
	±345	±992	±2039	±1128	±1446
Note: mean value ±S.E. (n=4)					

[0079] The aqueous preparations of compositions according to the invention exhibited average blood concentrations that increased as the sucrose fatty acid ester concentration was higher, from 0.1% (Example 20) to 0.25% (Example 19) to 0.5% (Example 18), with a constant bulk agent concentration, and therefore the absorption enhancing effect depended on the concentration of the sucrose fatty acid ester. For Example 19, the bioavailability was 33% as determined by the AUC obtained from the blood concentrations for 24 hours after administration.

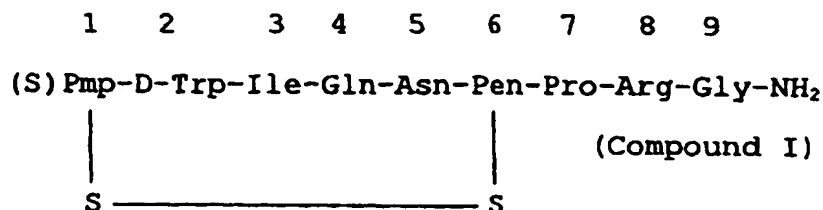
[0080] The blood concentrations increased as the bulk agent concentration was higher, from 0.25% (Example 19) to 0.5% (Example 21) to 1.0% (Example 22), with a constant sucrose fatty acid ester concentration.

Industrial Applicability

[0081] As explained above, a composition for permucosal administration according to the present invention is characterized by containing the physiologically active peptide Antago-3 or a physiologically acceptable salt thereof, and a sucrose fatty acid ester, and it has the high permucosal absorption of Antago-3 without irritation, rendering it useful as a long-term stable preparation.

Claims

1. A composition for permucosal administration characterized by containing the following compound I:

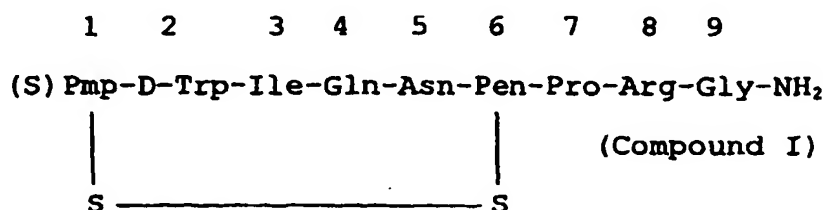


Pmp: β,β -(3-thiapentamethylene)- β -mercaptopropionic acid
D-Trp: D-tryptophan

Pen: L-penicillamine
 Ile: L-isoleucine Gln: L-glutamine
 Asa: L-asparagine Pro: L-proline
 Arg: L-arginine

or a physiologically acceptable salt thereof, and a sucrose fatty acid ester.

2. A composition for permucosal administration according to Claim 1, which contains the sucrose fatty acid ester in a proportion of 0.25-50 parts by weight to one part by weight of compound I or its physiologically acceptable salt.
3. A composition for permucosal administration according to Claim 1 or Claim 2, wherein the sucrose fatty acid ester is a sucrose stearic acid ester, sucrose palmitic acid ester, sucrose myristic acid ester, sucrose lauric acid ester or a mixture of two or more thereof.
4. A permucosal preparation characterized by containing the following compound I:



Pmp: β , β -(3-thiapentamethylene)- β -mercaptopropionic acid
 D-Trp: D-tryptophan
 Pen: L-penicillamine
 Ile: L-isoleucine Gln: L-glutamine
 Asn: L-asparagine Pro: L-proline
 Arg: L-arginine

or a physiologically acceptable salt thereof, a sucrose fatty acid ester, and benzalkonium chloride.

5. A permucosal preparation according to Claim 4, characterized by containing the sucrose fatty acid ester in a proportion of 0.25-50 parts by weight to one part by weight of compound I or its physiologically acceptable salt.
6. A permucosal preparation according to Claim 5, characterized by containing the benzalkonium chloride in a proportion of 0.03-0.5 part by weight to one part by weight of the sucrose fatty acid ester.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP98/01679

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl.⁶ A61K47/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl.⁶ A61K47/14

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category ^a	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, 5373089, A (Northwestern University), December 13, 1994 (13. 12. 94) & WO, 94025485, A	1-6
A	JP, 63-39822, A (Yamanouchi Pharmaceutical Co., Ltd.), February 20, 1988 (20. 02. 88) (Family: none)	1-6
A	JP, 2-214, A (Fujisawa Pharmaceutical Co., Ltd.), January 5, 1990 (05. 01. 90) (Family: none)	1-6
A	JP, 2-101020, A (Fujisawa Pharmaceutical Co., Ltd.), April 22, 1990 (22. 04. 90) (Family: none)	1-6

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.^a Special categories of cited documents:"A" document defining the general state of the art which is not
considered to be of particular relevance"E" earlier document but published on or after the international filing date
document which may throw doubts on priority claim(s) or which is
cited to establish the publication date of another citation or other
special reasons (as specified)"O" document referring to an oral disclosure, use, exhibition or other
means"P" document published prior to the international filing date but later than
the priority date claimed"T" later document published after the international filing date or priority
date and not in conflict with the application but cited to understand
the principle or theory underlying the invention"X" document of particular relevance; the claimed invention cannot be
considered novel or cannot be considered to involve an inventive step
when the document is taken alone"Y" document of particular relevance; the claimed invention cannot be
considered to involve an inventive step when the document is
combined with one or more other such documents, such combination
being obvious to a person skilled in the art

"Z" document member of the same patent family

Date of the actual completion of the international search
June 1, 1998 (01. 06. 98)Date of mailing of the international search report
June 9, 1998 (09. 06. 98)Name and mailing address of the ISA/
Japanese Patent Office

Authorized officer

Facsimile No.

Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.